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Auxin homeostasis during lateral root development under drought condition

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Lateral root formation is a critical agronomic trait in plant architecture that determines crop productivity and environmental stress adaptability. It is therefore tightly regulated both by intrinsic developmental cues, such as abscisic acid (ABA) and auxin, and by diverse environmental growth conditions, including water deficit and high salinity in the soil. We have recently reported that an Arabidopsis R2R3-type MYB transcription factor, MYB96, regulates lateral root meristem activation under drought conditions via an ABA-auxin signaling crosstalk. In this signaling scheme, the MYB96-mediated ABA signals are incorporated into an auxin signaling pathway that involves a subset of *GH3* gene encoding auxin-conjugating enzymes. The *MYB96*-overexpressing, activation tagging mutant, which is featured by having dwarfed growth and reduced lateral root formation, exhibits an enhanced drought resistance. In the mutant, expression of the *GH3* genes was significantly elevated, which is consistent with the reduced lateral root formation. In contrast, the *MYB96*-deficient knockout mutant produced more lateral roots and was more susceptible to drought stress. Our observations strongly support that *MYB96* is a molecular link that integrates ABA and auxin signals in modulating auxin homeostasis during lateral root development, particularly under water deficit conditions. It is also envisioned that the *MYB96*-mediated signals are related with pathogen resistance response, which is also profoundly affected by water content in plant cells.

Key words: arabidopsis, abscisic acid, auxin homeostasis, lateral root, MYB, GH3, drought

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The MYB transcription factors, consisting of approximately 170 members, comprise one of the largest transcription factor families in the Arabidopsis genome.[1] They are classified into three subfamilies by the number of adjacent sequence repeats in the MYB domain. Approximately 70% of the MYB members have two imperfect repeats (R1 and R2), each repeat containing ~50 residues that form a helix-turn-helix configuration, belong to the R2R3-type subfamily in Arabidopsis.[1,2] The R2R3-type MYB members regulate a wide array of plant developmental processes and plant responses to environmental stresses, such as anthocyanin accumulation,[3] secondary metabolism,[4] epidermal cell patterning[5,6] and abiotic stress signaling.[7-9]

Numerous transcription factors have been found to mediate drought resistance responses. Transgenic or mutant plants with altered expression of such drought-inducible genes exhibit disturbed stomatal aperture and thus distressed response to water deficit conditions. However, only a few transcription factor genes have been shown to regulate root formation under drought conditions. It is therefore notable that the MYB96 transcription factor plays a role in lateral root growth under drought stress.

We have isolated an activation tagging mutant that constitutively overexpresses the *MYB96* gene from a T-DNA insertional Arabidopsis mutant pool. The *MYB96*-overexpressing mutant exhibits an enhanced drought resistance with reduced lateral root density. While the primary root growth is unaffected in the mutant, the number of lateral roots is significantly reduced. Consistent with this phenotype,

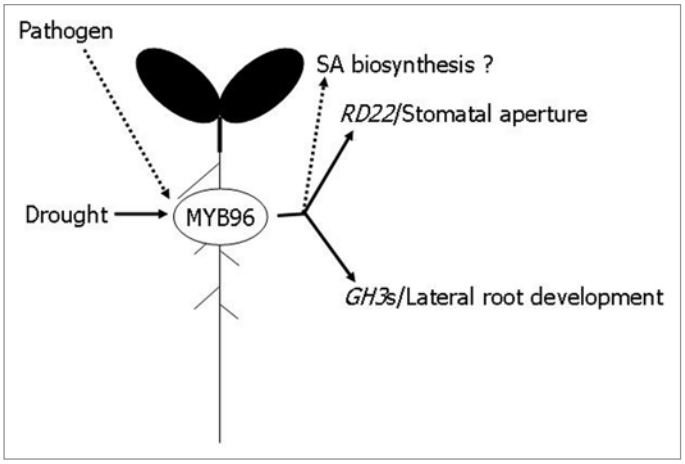

Figure 1. A proposed working model for MYB96 function in plant response to biotic and drought stresses. Regulation of stomatal aperture is modulated by the *MYB96*/*RD22*-mediated pathway in the shoots. *MYB96* also regulates auxin metabolism by inducing the *GH3* genes to optimize root growth under drought conditions. In addition, *MYB96* may play additional roles in disease resistance response by regulating SA biosynthesis.

the *MYB96* gene is expressed to a high level in the lateral root primordia. More careful examination revealed that reduced lateral root number in the mutant is caused by arrested activation of the lateral root meristem. In addition, while lateral root initiation and establishment of lateral root primordia are essentially normal, lateral root elongation is significantly suppressed. These phenotypes are also observed in plants treated with ABA or grown under osmotic stress.[10,11] The previous and our own observations support that lateral root development is closely related with ABA and drought stress.

Whereas suppression of lateral root development is a well-known response to drought stress, not all of the mutants that are resistant to drought stress exhibit reduced lateral root number. For example, the *enhanced drought tolerance 1* (*edt1*) mutant has an enhanced root system with longer primary root and more lateral roots.[12] It is therefore likely that there are extensive signaling crosstalks in regulating root development under drought stress.

Our data support that the *MYB96* gene plays inter-related but distinct in the shoots and roots under drought conditions. While ABA induces the *MYB96* gene both in the shoots and roots, auxin induces the *MYB96* gene primarily in the roots. Furthermore, *MYB96* regulates different targets differentially in different organs: it regulates *RD22* in the shoots but regulates primarily a subset of the *GH3* genes in the roots in response to drought stress. Although ABA induction of the *GH3* genes is unaffected in the shoots of the MYB96-deficient mutant (*myb96-1*), it is significantly diminished in the roots of the *myb96-1* mutant in response to ABA. It is interesting that a transcription factor plays dual roles in different organs.

To look into the functional relationship of *MYB96* with ABA and auxin, we investigated its expression patterns in response to exogenous ABA and IAA applications. Unlike in the plants treated with ABA, *MYB96* transcripts were accumulated throughout the whole root system, including the vascular tissue in the IAA-treated plants. Detailed analysis revealed that *MYB96* expression is initiated in the pericycle, the initiation site of lateral root formation. The expansion of *MYB96* expression domain in the presence of auxin might be closely related with the promotive effects of auxin on the lateral root development.

When plants were treated with both ABA and IAA, the level of *MYB96* transcript was lower than that observed in the IAA-treated roots, indicating that ABA inhibits the action of IAA on the pericycle cell division. Accordingly, less lateral root primordia were produced in the presence of IAA and ABA, which on its turn results in less cells expressing *MYB96*. It is therefore suggested that IAA and ABA act antagonistically and ABA is epistatic to auxin in certain lateral root developmental stages.

Linking with auxin homeostasis is a prominent point in the *MYB96*-mediated ABA signaling pathway. The *MYB96* regulation of the *GH3* genes is restricted to the roots, particularly in the lateral root primordia, suggesting that reduced lateral root number is caused by altered auxin metabolism. Auxin is a well-known regulator functioning throughout the whole lateral root growth and developmental process.[13] In addition, auxin plays important roles in cell division and establishment of the lateral root meristem during the lateral root emergence stage.[14,15] Recent studies have also suggested that extensive ABA-auxin interactions take places during the lateral root emergence stage.[10] The promotive effects of auxin on the lateral root meristem activation might be inhibited by the repressive actions of ABA.[15] Indeed, lateral root formation of the *MYB96*-overexpressing mutant is suppressed in the lateral root emergence stage, and this lateral root phenotype is not rescued by exogenous application of auxin. In this regard, the *MYB96*-mediated regulation of auxin metabolism to inhibit lateral root growth is an interesting example showing how ABA-auxin crosstalks occur during the lateral root development.

Web-based global gene expression analysis data suggest that *MYB96* might also have a role in pathogenesis. When plants are treated with pathogen elicitor, such as bacterial flagellin peptide elicitor (flg22), oligogalacturonides (OG) and chitin, *MYB96* expression is significantly induced. We recently found that endogenous level of salicylic acid (SA) and expression of SA biosynthetic genes are highly upregulated in the shoots of the *MYB96*-overexpressing mutant shoots (data unpublished). It is possible that *MYB96* may also function as a molecular link that mediates ABA-SA crosstalks in the shoots (**Fig. 1**).

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